Plasma Leptin Concentrations in Patients with Stable Chronic Obstructive Pulmonary Disease

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Abstract

Unexplained weight loss is common in patients with chronic obstructive pulmonary disease (COPD). Increased circulating of tumor necrosis factor-α (TNF-α) and leptin concentrations were reported in weight-losing COPD patients. Leptin is known to play important roles in the control of body-weight and energy expenditure. The aim of this study was to investigate circulating serum leptin and TNF-α levels in patients with COPD, and to determine whether serum leptin levels are related to body mass index (BMI) or to TNF-α levels. Serum concentrations of leptin, TNF-α, albumin and creatinine clearance (C_{Kr}) were measured in 32 male patients with COPD and 18 healthy control subjects. Patients with COPD had significantly lower BMI, leptin, albumin and C_{Kr} levels than did the control subjects. Serum TNF-α levels in COPD patients were significantly high compared to the healthy controls. Circulating leptin levels did not correlate with BMI and TNF-α neither in COPD patients or in healthy controls.

These data suggest that circulating leptin is decreased and TNF-α levels increased in patients with stable COPD. In addition, circulating leptin works independently of the TNF-α system and does not primarily affect BMI in COPD patients.

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Introduction

The frequent occurence of weight loss and subsequent tissue depletion in patients with chronic obstructive pulmonary disease (COPD) is associated with an increased morbidity and even mortality (1). Weight loss may involve all tissue compartments, although loss of skeletal muscle may be particularly important because of wasting of respiratory muscles with loss of power and endurance (2). The increased work of breathing in COPD could be partly responsible for excessive energy expenditure (3). An elevated energy metabolism not adequately met by an increased spontaneous dietary intake underlies weight loss in COPD (4). Inflammatory activity probably contributes to catabolic processes (5).

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In a recent study of COPD patients in a stable phase, it was reported that the basal metabolic rate is related to plasma concentrations of tumor necrosis factor a (TNF-α), but not to respiratory function (6). It was shown that both circulating levels of TNF-α and TNF-α production by peripheral blood monocytes were increased in weight-losing COPD patients (7). Administration of endotoxin or cytokines including TNF-α or IL-1 produced a prompt and dose dependent increase in serum leptin levels in experimental studies (8, 9). A recent study suggests that leptin, a protein synthesized by adipose tissue and encoded by the *ob* gene (10), plays an important role in energy balance. Leptin regulates the energy balance through a feedback mechanism in which the hypothalamus is involved and administration of leptin results in a reduction in food intake as well as in an increase in energy expenditure (11). The increased levels of circulating leptin may be one mechanism by which anorexia is induced during acute inflammatory conditions such as those which may occur in COPD. In stable depleted patients with COPD, dietary intakes as well as weight gain after nutritional therapy was found to be inversely related to plasma leptin (12).

Several plasma proteins of hepatic origin, such as albumin, prealbumin and retinol binding protein have been suggested to be good dynamic indices of nutritional status (13). Changes in the plasma concentration of these proteins may reflect changes in nutritional status (14). Twenty-four-hour urinary creatinine excretion is another biochemical marker of nutritional status which correlates well with total body muscle mass in individuals with normal renal function (13).

Our study was undertaken to investigate nutritional status in patients with COPD by measurement of body mass index (BMI) and biochemical markers of nutrition. In addition, we investigated the questions of: 1) whether circulating leptin levels are inappropriately increased in patients with COPD, and 2) whether circulating levels of leptin are related to those of TNF-α in patients with COPD.

Materials and Methods

The patient group, consisted of patients consecutively admitted to the Chest Diseases Department of the Faculty of Medicine of Fırat University.

Thirty two patients with COPD (all males) were diagnosed according to the criteria of the American Thoracic Society (15). Presence of irreversible chronic airflow obstruction was confirmed by spirography. Chronic airflow obstruction was defined as a measured forced expiratory volume in one second (FEV_1) less than 80% of the predicted value, irreversible obstructive airway disease, i.e., <12% improvement in FEV_1 expressed as percentage of predicted after inhalation of a β2-agonist. All patients with COPD had been clinically stable for at least 3 months prior to admission and were free of clinical signs of exacerbation, such as infection or heart failure. Patients who had conditions known to affect serum leptin or TNF-α levels, such as use of systemic corticosteroids, cancer, collagen vascular disease, smoking, cardiac failure and infection were excluded (16-20). The patients were not receiving any nutritional support therapy. All patients were receiving inhaled β2 agonists, inhaled ipratropium bromide, inhaled corticosteroid treatment.

Eighteen age-matched healthy volunteer males were also studied as control subjects. All control subjects had no history of endocrine, renal, liver, or other metabolic disorder, were free of any ailment and had no pathology detected by physical examinations or routine laboratory data. All were non-smokers.

Informed consent was obtained from all subjects in the study.

Anthropological indices

Height was determined to the nearest 1.0 cm with subjects standing barefoot. Body weight was measured on the same scale in all subjects, with subjects in their underwear and wearing no shoes. BMI and % fat were calculated as below:

BMI=body weight/height^2 (weight in kg and height in meter units) BMI values of <20 were accepted as underweight (13). % fat = [(1.2xBMI) + (0.23xage)] - [(10.8xgender)-5.4] (male:1, female:0) Ages of all subjects were recorded.

Biochemical nutrition parameters

The creatinine clearance was determined by measuring 24-h urinary creatinine and serum creatinine and calculating creatinine clearance. Clearance values between 80 and 139 mL/min were accepted normal (13, 21).

Serum albumin levels were assessed by an Olympus AU 600 autoanalyzer. Normal range for albumin: 3.5-5.5 g/dL.

Determination of serum leptin and TNF levels

Blood samples were drawn in the early morning (8:30 AM), when patients were in the fasting state for at least 10 hrs. Both serum and plasma were separated from blood cells by centrifugation at 1 000 x g for 5 min. All samples were stored at-70°C until analyzed.

Serum leptin levels were determined with enzyme linked immunosorbent assay (ELISA) kits (DRG, DRG instruments GmbH, Germany).

Serum TNF-α levels were measured with ELISA kits (Cytoscreen Biosource int. Camarillo CA USA) according to the manufacturer's instructions. All analyses and calibration were performed in duplicate.

Lung function

Spirometric tests were performed according to national guidelines using a "Fukuda Denshi Spirosift 500" equipment (22, 23).

Degree and reversibility of airway obstruction were assessed according to GOLD (24). Indices of airflow obstruction, FEV_1 and FEV_1/FVC were measured. FEV_1 and FVC were expressed as percentage of predicted values (FEV_1% and FVC%) according to the prediction equation of the European Respiratory Society (25).

Blood was drawn from the radial artery at rest while breathing room air. Arterial pressures of oxygen (P_{O2}) and carbon dioxide (P_{CO2}) were analyzed on a pH/blood gas analyzer (Rapid lab 348, Biobak, Chiron, Bayer Diagnostic, UK).

Statistical analysis

Statistical analysis was performed with the Mann-Whitney U Test for nonparametric data to analyze differences between the two groups. The relations between continuous variables were evaluated with Spearman's correlation test. Results were expressed as median (range). Significance was determined at the 0.05 level. Statistical analysis was done with the SPSS 10.0 package programmes.

Results

Characteristics of both COPD patients and healthy controls and their serum leptin, TNF-α, albumin concentrations, C_{Kr}, P_{O2}, P_{CO2} levels are given in Table 1.

Patients with COPD had significantly lower body weights and lower BMI, leptin, albumin and C_{Kr} levels than did the control subjects. COPD patients also had decreased arterial P_{O2}, and increased arterial P_{CO2} values. The levels of FEV_1 (% pred) and FVC (% pred) were significanlty lower in COPD patients than in healthy controls. Serum TNF-α levels in COPD patients were significantly higher than those in the healthy controls.

There was no correlation between serum leptin levels and BMI in patients with COPD and in healthy subjects, too. (Figure 1A-B).

Serum leptin levels did not correlate with serum TNF-α in either the COPD patients or the healthy controls (Figure 2A-B).

Characteristics of COPD sub-groups defined by BMI

BMI was low (<20 kg/m²) in 9 COPD patients. Mean value for BMI was 17.69±1.50 kg/m² in this subgroup compared with 23.93±3.86 kg/m² in the remaining COPD patients (p<0.000). Characteristics and concentrations of serum leptin, TNF-α, albumin, C_{Kr} in COPD patients who had low BMI and in the remaining COPD patients are shown in Table 2.

Patients with COPD who had low BMI were characterized by a significantly lower FEV_1 compared with the remaining COPD patients. We found that COPD patients who had low BMI showed decreased serum leptin and increased serum TNF-α levels compared to the remaining COPD patients, but the differences were not of statistical significance.

Table 1. Patient characteristics and concentrations of serum leptin, TNF-α, albumin and creatinine clearance in patients with COPD and healthy subjects

	COPD patients (n=32)	Healthy controls (n=18)	p Value*
Age, yrs	65 (41-81)	60 (46-83)	NS
Height, m	1.65 (1.45-1.84)	1.73 (1.55-1.82)	< 0.046
Weight, kg	60 (39-90)	74 (58-93)	< 0.000
BMI, kg/m²	21.48 (15.22-35.13)	24.68 (20.48-30.39)	< 0.02
% fat	34.82 (27.35-54)	33.87 (28.02-48.17)	NS
FVC, % pred	46.50 (34-80)	99.50 (92-110)	< 0.000
FEV_1, % pred	39 (22-83)	102 (95-115)	< 0.000
Pa_{O2}, mm Hg	55.80 (38.40-85)	ND	-
Pa_{CO2}, mm Hg	42.55 (30.90-64)	ND	-
Leptin, ng/mL	1.73 (0.12-7.69)	7 (2.20-18)	< 0.000
TNF-α, pg/mL	5.49 (5.04-6.04)	0.74 (0.1-3.80)	< 0.000
Albumin g/dL	3.85 (2.87-4.80)	4.96 (4.42-5.76)	< 0.000
C_{Kr}, mL/min	49.67 (9.19-151)	75.83 (49.75-102.30)	< 0.027

Definition of abbreviations: C_{Kr}; creatinine clearance, BMI; body mass index, ND; not determined, NS; non significant. Values presented are medians and ranges (in parenthesis).
* Mann Whitney U Test

Table 2. Characteristics and measurement of parameters in COPD patients with low BMI and the remaining COPD patients			
	COPD patients (n=32)		
	BMI < 20 kg/m² (n=9)	BMI ≥ 20 kg/m² (n=23)	p Values*
Age, yrs	67 (51-73)	65 (41-81)	NS
FEV_1, % pred	29 (22-54)	42 (28-83)	< 0.03
Pa_{O2}, mm Hg	55 (42.90-79.40)	55.90 (38.40-85)	NS
Pa_{CO2}, mm Hg	41 (30.90-59)	43 (31.80-64)	NS
Leptin, ng/mL	0.97 (0.29-3.94)	2.59 (0.12-7.69)	NS
TNF-α, pg/mL	5.56 (5.04-6.04)	5.48 (5.10-5.92)	NS
Albumin g/dL	3.70 (2.87-4.20)	3.86 (2.90-4.80)	NS
C_{Kr}, mL/min	36.49 (9.19-103.12)	52.38 (22-151)	NS

Definition of abbreviations: C_{Kr}; creatinine clearance, NS; non significant. Values presented are medians and ranges (in parenthesis)
* Mann Whitney U Test

Discussion

In this study, the possible associations between circulating leptin levels and TNF-α, and other nutritional parameters such as albumin and C_{Kr} in COPD patients and in healthy subjects were investigated.

The mechanisms underlying the metabolic changes associated with the wasting process are complex. They involve interaction between cytokines and the hypothalamus and the direct effects of IL-1 and TNF-α on peripheral tissues and liver (26). TNF-α, a multifunctional cytokine, may play a potential role in the weight loss noted in COPD patients as well as in other cachectic patients with chronic wasting diseases (7, 27). As expected, we found that serum TNF-α levels in COPD patients were elevated compared with healthy controls. Since no direct correlations between circulating TNF-α levels and weight loss were found in patients with COPD, unidentified factors that could interfere with both the TNF-α system and cachexia in COPD patients have been considered (7). There are several speculations for the increased TNF-α levels in COPD patients. First, inflammation is the source of activation of the TNF-α system (26). Second, hypoxemia might contribute to the activation of the TNF-α system independently of airways inflammation (16). Third, the increased levels of circulating TNF-α could be related to the pathophysiology of right-sided heart failure associated with COPD (16). It has been demonstrated that serum levels of TNF-α were much higher in weight losing COPD patients than in COPD patients of normal weight (28, 29). These studies have shown that impairment of lung function and hypoxemia tended to be more severe in cachectic patients with COPD than in noncachectic patients with COPD. In the present study, there were no differences in TNF-α levels between COPD patients with low BMI (<20 kg/m²) values and the remaining COPD patients, while a significant difference was found between COPD patients and healthy controls. A possible explanation for the absence of any difference in TNF-α values of COPD patients with a low BMI (<20 kg/m²) and TNF-α values of remaining COPD patients in our study could be that both groups of patients had similar levels of hypoxemia. These findings can also be interpreted to indicate that increased levels of TNF-α are not associated with BMI and/or cachexia in patients with COPD, although the numbers of COPD patients in each subgroup were relatively small.

Against expectation, we found that serum leptin levels were decreased in COPD patients compared with healthy controls, although serum TNF-α levels were significantly increased in the COPD patients. There is also a relationship between inflammatory cytokines, such as TNF-α or IL-1, and leptin in inflammatory state. Leptin levels may be one mechanism by which anorexia is induced during acute inflammatory conditions (9). The normal leptin feedback mechanism can be disturbed by several factors. In animals, administration of endotoxin, TNF-α or IL-1, inflammatory cytokines known for their anorectic effects, resulted dose dependently in an increase in circulating leptin concentrations (8, 9). Several studies conflicting results on the relationship between serum leptin and TNF-α levels. For example, Schools et al showed that a significant relationship existed between these parameters in patients with emphysema (12). In contrast, Takabatake et al demonstrated that serum leptin levels did not correlate with serum TNF-α levels (16). In our study, we demonstrated that there was no correlation between serum leptin and TNF-α levels in patients with COPD and in healthy subjects. These observations suggest that leptin is not primarily under the control of the TNF-α system.

Serum leptin concentrations are correlated with the percentage of body fat and with BMI, suggesting that most

obese persons are insensitive to endogenous leptin production (30). In humans, there was variability in plasma leptin levels at each BMI group, suggesting that there are differences in its secretion rate independent of body fat. However, weight loss due to food restriction was associated with a decrease in plasma leptin in samples from obese humans (31). Low leptin levels in COPD patients may develop secondary to weight loss. In our study, we showed that there was no correlation between serum leptin levels and BMI neither in COPD patients nor in the healthy controls. These results suggest that, serum leptin levels do not primarily affect BMI and decreased serum leptin levels may not be associated with weight loss in COPD patients.

The advantage of analyzing plasma proteins in the assessment of nutritional status is that their concentrations are not influenced by body weight (32). Malnutrition could be detected in patients with COPD while they still have normal body weight. Albumin levels have been recommended as a means of detecting and monitoring protein calorie malnutrition, because levels vary directly with adequacy of intake (13). Other than protein calorie malnutrition, the most common cause of low albumin levels is acute and chronic inflammation states. Plasma albumin concentrations were found to be similar in COPD patients and control groups (32). It was also reported that BMI and albumin significantly underestimated the prevalence of malnutrition in hospitalized patients compared with body composition measurements (33). As biochemical markers of malnutrition, we analyzed plasma albumin and twenty-four-hour urinary creatinine in COPD patients and found that, they had decreased plasma albumin levels and creatinine clearance values compared with healthy subjects. However, there were no differences in plasma albumin levels between COPD patients with a low BMI (<20 kg/m^2) and the remaining COPD patients.

Available information suggests that muscle wasting is present in a large population of patients with COPD but its prevalance can only be approximated as there are no simple techniques to measure muscle mass (34). The BMI was of limited value for determining changes in body composition and did not identify patients with skeletal muscle depletion. Twenty-four-hour urinary creatinine excretion levels correlate well with total body muscle mass in individuals with normal renal function (13). In one study, skeletal muscle depletion was reported in 41% of 80 patients with COPD when evaluated with creatinine height index (35). In our study, patients with COPD had significantly lower body weights, lower BMI values and lower creatinine clearance levels than the control subjects.

In conclusion, we demonstrated that BMI, serum albumin and C_{Kr}. values were decreased in patients with COPD. In addition, circulating levels of leptin were significantly lower in patients with COPD compared to healthy controls. Circulating leptin levels were not correlated with BMI. These data suggest that circulating leptin is decreased and TNF-α levels are increased in patients with stable COPD. In addition, circulating leptin works independently of the TNF-α system and does not primarily affect BMI in COPD patients.

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